76. The method according to claim 69 wherein the PP2Cα gene activity is defined by assaying PP2Cα gene product including polymorphisms thereof in the cells with an assay selected from the group consisting of immunohistochemical and immunocytochemical staining, ELISA, RIA, immunoblotting, immunoprecipitation, Western blotting, functional assays and protein truncation test.

REMARKS

Reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Applicant has again amended previously amended claim 65, and has added new claims 68-76, inclusive. The amendments to the claim and the new claims are all fully supported in the present specification as follows:

Claim 65 has been amended to further define the cancerous cells and the vector. This is supported in the present application at p. 10 lines 21-25 and at p. 16 lines 6-10, respectively.

Claim 49 has been cancelled and substituted by new claim 69. Claim 69 defines a method of introducing a vector into cancerous cells, which includes a step of determining the type of cancer and cancerous cells wherein a decrease in PP2Cα gene activity compared to normal cells is detected. This is supported in the present specification at p. 10 lines 21-25.

Claim 71 has been added to define the active steps for introducing the vector into cancerous cells. This is supported in the present specification at p. 16 lines 14-26.

Claims 72 to 74 have been added to define the vector, which includes at least one targetor moiety. This is supported in the present specification at p. 16 line 35 through p. 17 line 2.

Claim 75 has been added to define PP2Cα gene activity by assaying mRNA complementary to PP2Cα DNA including polymorphisms thereof in the cells. This is supported in the present specification at p. 11 lines 20-25.

Claim 75 has been added to further define PP2Cα gene activity by assaying PP2Cα gene product including polymorphisms thereof in the cells. This is supported in the present specification at p.11 lines 26-35.

The rejections to claims 49, 50 and 65 under 35 U.S.C. 112, first paragraph, have been overcome by the amendment. Accordingly, it is solicited that the rejection be withdrawn.

The rejections of claims 49 and 50 under 35 U.S.C. 112, second paragraph, for indefiniteness have been overcome by the amendment and, accordingly, their withdrawal is respectfully solicited.

Prior to addressing the specific rejections, it should be noted that the essential novelty and non-obviousness of the claimed invention is that for the first time a method of treating cancer in a patient by gene therapy in which PP2Cα is expressed has been taught.

The Examiner has rejected claims 49, 50 and 65 as containing subject matter which was not described in the specification under 35 U.S.C. 112, first paragraph. The rejection is respectfully traversed.

The Examiner agreed that the expression of PP2Cα is decreased in colorectal tumor samples but indicated that "the instant claims encompass the treatment of any type of cancer" and added that "the specification teaches that samples from other tissues show no or a varying change of expression and location of the PP2Cα in the cells tested (Example 4)" (see p. 3 last paragraph of the Office Action).

It is respectfully submitted that the decrease in PP2Cα in cancerous cells compared to normal cells has been delineated in the specification as follows: "In general, it is found in cell transformation as shown in the Examples herein below (Examples 4, 5) that the activity/expression of PP2Cα is reduced compared to that of normal controls as determined by a reduction in the amount of gene product in the cell" (p. 10 lines 21-25).

The present application describes the decrease of PP2Cα gene activity in 3 different tumors. One of ordinary skill in the art could use these exemplary tumors to extend the method of the present invention to any other type of cancer or a given cancer in any specific patient may be done by one skilled in the art. Applicant would like to note that the claims herein have been amended to encompass a method of treating cancer in a patient which comprises administering to the patient a vector

comprising an expression control sequence operatively linked to the nucleic acid sequence of mammalian PP2Cα, said vector capable of targeting the cancerous cells *wherein a decrease in PP2Cα gene activity is detected.* Support for the amendments can be found at p.10 lines 21-25.

In Example 4 of the present specification, Applicant describes the expression of PP2Cα in breast tumor and in hepatoma. It is shown that while the nuclei in normal samples of the breast were stained very predominantly with antibodies specific to PP2Cα, no staining of the nuclei was observed in invasive carcinoma samples using the same antibodies (Example 4). Similarly, a pronounced cytoplasmic staining with few very strong nuclear regions was observed in normal liver while in hepatoma the cytoplasma was faintly stained and less staining in the nucleus was observed.

Thus, the expression of PP2Cα gene activity was consistently lower in the tumors tested in the present application (colorectal tumor, breast tumor and hepatoma; see Examples 4 and 5) than in the normal tissue. In line with the instant application, Saadat et al. (1994) conducted a study describing the gene expression of protein phosphatases 1α, 1γ1, 2Aα and 2Cα in rat ascites hepatoma cell lines and indicated that the expression of PP2Cα decreased (at a range of 0.23-0.9) in 13 out of 14 ascites hepatoma cells compared to normal rat liver. The decrease in PP2Cα expression was found to be of the smallest magnitude in the AH-13 cell line (0.9 compared to normal liver; see Table 1 and Fig. 1 in Saadat et al., 1994).

The observations raised by the Examiner in his Office Actions of October 01, 2001 (see page 3) and of May 02, 2001 (page 5) that "when Kitamura et al. examine and compare normal liver tissue samples and AH13, a rat hepatoma cell line, they also see no change in PP2Cα expression levels" relates to only one specific ascites hepatoma cell line, i.e, the AH-13 in which a decrease in PP2Cα expression was small, while in most other cell lines, i.e., indeed in 12 out of 13 lines tested by Saadat et al. (1994) the decrease in PP2Cα was found to be very pronounced.

In addition, Kikuchi et al. (Cancer Det. Prevent. 21: 36-43, 1997; after the date of the present invention) showed that the mRNA level of PP2C, like that of PP1α and of PP2A, increased in chemical hepatocarcinogenesis (Fig. 1) but indicated that in primary hepatoma, the PP2C mRNA was found to be lower than that of the control livers (page 39, end of 1st paragraph). This is in accordance with the findings of Kitamura et al. (1992), demonstrating that in a chemical hepatocarcinogenesis model

in rat the mRNA levels of PP1α, PP2A, and PP2C were elevated 8, 29 and 11 times, respectively, as compared to those of the control livers. However, in primary hepatoma induced according to the Solt-Farber model, the mRNA levels of all three protein phosphatases were dramatically decreased to normal levels or even to much lower levels, according to the same reference.

The examiner has interpreted the above observations erroneously citing that "various types of cancer do not show changes in PP2Cα expression when tested" and also "Kitamura et al. in analyzing protein phosphatases 1, 2A and 2C in hepatocarcinogenesis, demonstrate that while other phosphatases increase in expression levels in a Solt-Farber model the level of PP2Cα is unaltered" (Office Actions of October 01, 2001, page 3 and of May 02, 2001, page 5, respectively).

Based on the present specification, claims 49 and 50 have been withdrawn and replaced by new claims 69-76 and claim 65 has been amended to encompass cancer cells in which a decrease in PP2Cα gene activity compared to normal cells has been detected.

The Examiner indicated that "Applicants argue that expression of PP2Cα can alter the transformed phenotype of a cell, however while a review of the data in example 7.....the data suggests that it is possibly the integration of the AAV, not PP2Cα expression which is important for the transformed phenotype" (see Office Action, page 4). The present application discloses the role of PP2Cα in the initiation and/or maintenance of SV40 transformed phenotype (p. 25 line 15 through page 34 line 8). Applicant uses SV40 transformed Chinese hamster embryonic cells in which AAV/neo virus was introduced. Analysis of PP2Cα mRNA indicated that treatment of AAV/neo SV40 transformed cells with DNA damaging agents reduced PP2Cα mRNA while identical treatment to the parental SV40 transformed cells increased PP2Cα mRNA. Moreover, stable transfection of the AAV/neo cells (which lost the transformed phenotype following AAV integration) with the PP2Cα cDNA resulted in regaining the transformed phenotype. Applicant concluded that "the fact that inactivation of only one allele of PP2Cα is responsible for the changes in the transformed phenotype and the introduction of a functional PP2Cα cDNA clone rescues the transformed phenotype demonstrates the importance of PP2Cα" (p. 33 line 36 through page 34 line 5).

Thus, although the results presented in Example 7 suggest that the AAV silencing elements play a role in the transformed phenotype, PP2Cα plays a crucial role as well. This role of PP2Cα is described both for SV40 transformed Chinese hamster embryonic cells and for mouse mammary tumor cells (p. 25 line 15 through p. 34 line 8 and Example 1). Such a role may be applicable for human cancer cell lines as well.

The Examiner refers to the need for the specific guidance required to practice the instant invention and indicates that "the experiments pointed to for support of an enabling disclosure are done *in vitro* with cell lines in culture" (Office Action page 4). However, the Examiner adds further that "though working examples are not required to provide an enabling disclosure, because of the unpredictability of gene therapy protocols recognized in the art detailed guidance to the specific types of cells to be targeted for gene expression and a means to target said cells, detailed guidance to the required levels of expression of the inserted gene and a means to obtain and control said levels of expression are required" (page 4).

The present specification discloses detailed guidance for the selection and methods of using vectors in order to alter PP2Cα expression in cancer cells (p.16 line 6 through p. 21 line 15). For example, it describes the use of adenovirus derived vector to infect cells having adenovirus receptor, which is present in most cancers of epithelial origin (p. 17 line 34 through p. 18 line 6). Moreover, the response filed on July 05, 2001, provided several references describing genomic targeting of a vector, which allowed highly reproducible gene expression in mammalian cells. Specifically, Fukushige and Sauer (1992) described the use of the Cre-*lox* site-specific recombination machinery to construct isogenic cell lines, thus allowing reproducible gene expression in stably transformed CHO cells.

As an additional reference, the response filed on July 05, 2001 referred to the FDA approved protocol for the administration of an antisense drug for treating CMV infections in the eye. It should be noted that this reference was provided as an example for successful gene therapy that is currently available as a method of treatment in CMV infections in the eye. There was no intention in referring to this protocol to indicate that administration of antisense PP2Cα oligonucleotides as the method of treatment in the present specification, but rather to emphasize that there is specific knowledge, with regard to gene therapy, which is currently available and that those of skill in the art would view gene therapy as a reproducible and successful

method of treatment. The present application encompasses claims for a method of treating cancer in a patient using vectors encoding PP2Cα in order to increase PP2Cα gene activity.

It should be pointed out that many reviews summarizing gene therapy protocols for cancer treatment were available prior to the filing date of the present application. For example, Geraghty, P.J, and Chang, A.E. (Surg. Oncol. 4: 125-137, 1995); Altaner, C. (Neoplasia 42: 209-213, 1995); Vile, R., and Russel, S.J. (Gene Ther. 1: 88-98, 1994); Parmiani, G., and Colombo, M.P. (Melanoma Res. 5: 295-301, 1996); Cooper, M.J. (Semin. Oncol. 23:172-187); Berns, K.I., and Girand, C. (Ann. N.Y. Acad. Sci. 772: 95-104, 1995). These reviews focus on different aspects of gene therapy in cancer, on the use of viral-based vectors as well as non-viral vectors, and assess the main clinical trials of gene therapy for cancer.

In his conclusions of the Office Action the Examiner indicated that Claims 49, 50 and 65 are free of the art of record because the art fails to teach a method of treating cancer in a mammal by gene therapy protocols in which PP2Cα is expressed. Indeed, the present application discloses a new method of treating cancer making use of gene therapy wherein PP2Cα nucleic acid sequences are introduced into cancer cells. The specification discloses prior art for gene therapy with the notion that these methods rely on gene delivery protocols established by others and, hence, do not require elaboration. The novelty and non-obviousness of the claimed invention is the use of PP2Cα gene in treating cancer in a patient.

The Examiner indicates that " The instant specification....fails to provide a nexus between a proposed role of PP2Cα expression in transformed cells with the necessary guidance for the skilled artisan to alter said expression such that any treatment is achieved" (p. 5 in the Office Action). Recently, the present inventor and her coworkers reported the use of conjugates of PP2Cα as a specific intracellular antitumor drug (Satchi-Fainaro et al., 28th Intern. Symp. on: Controlled Release of Bioactive Materials, June 23-27th, 2001, San Diego, USA). The results indicated that administration of PP2Cα conjugate to C57 black male mice bearing subcutaneous B16F10 murine melanoma significantly reduced tumor growth rate. These results support the potential use of PP2Cα in cancer biotherapy, and may be provided in the form of a declaration under section 132 if required.

D'vorah Graeser, Reg No 40,000, unsigned! informal!